



## Changes of pH in -Lactoglobulin and -Casein Solutions during High Pressure Treatment

Olsen, Karsten; Jespersen, Bo Bindzus; Orlie, Vibeke

*Published in:*  
International Journal of Spectroscopy

*DOI:*  
[10.1155/2015/935901](https://doi.org/10.1155/2015/935901)

*Publication date:*  
2015

*Document version*  
Publisher's PDF, also known as Version of record

*Citation for published version (APA):*  
Olsen, K., Jespersen, B. B., & Orlie, V. (2015). Changes of pH in -Lactoglobulin and -Casein Solutions during High Pressure Treatment. *International Journal of Spectroscopy*, 2015, [935901].  
<https://doi.org/10.1155/2015/935901>

## Research Article

# Changes of pH in $\beta$ -Lactoglobulin and $\beta$ -Casein Solutions during High Pressure Treatment

Karsten Olsen, Bo B. Jespersen, and Vibeke Orlien

Food Chemistry, Department of Food Science, Faculty of Science, University of Copenhagen, Rolighedsvej 30, 1958 Frederiksberg, Denmark

Correspondence should be addressed to Vibeke Orlien; vor@food.ku.dk

Received 29 October 2014; Accepted 9 February 2015

Academic Editor: Guang Zhu

Copyright © 2015 Karsten Olsen et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The pH changes in the milk systems,  $\beta$ -lactoglobulin B,  $\beta$ -casein, and mixture of  $\beta$ -lactoglobulin and  $\beta$ -casein (pH 7 and ionic strength 0.08 M) were measured *in situ* during increasing pressure up to 500 MPa. An initial decrease to pH 6.7 was observed from 0.1 to 150 MPa for  $\beta$ -lactoglobulin, followed by an increase to pH 7.3 at 500 MPa. The initial decrease is suggested to be caused by the deprotonation of histidine, while the increase is suggested to result from an increase of hydroxide ions due to the loss of tertiary structure. The change in pH of the  $\beta$ -casein solution displayed an almost linear increasing pressure dependency up to a pH of 7.7 at 500 MPa. The limited tertiary structure of  $\beta$ -casein could allow exposure of all amino acids; thus the increase of pH can be caused by binding of water protons resulting in an increase of hydroxide ions. Addition of  $\beta$ -casein to  $\beta$ -lactoglobulin (1:1) was found to suppress the initial pH decrease found for the  $\beta$ -lactoglobulin solution. The pH change of the mixture did not suggest any intermolecular interaction, and a simple additive model is proposed to calculate the pH change of the mixture from the corresponding individual samples.

## 1. Introduction

Controlling the pH during the various steps during production of dairy products is crucial for the product quality. As reviewed by Salaün et al. [1] the buffering capacity of the product plays a major role in the variations in pH. Regarding cow's milk the caseins, whey proteins, soluble minerals, and colloidal calcium phosphate (CCP) contribute 35%, 5%, 40%, and 20%, respectively, to milk's buffering capacity [1]. The pH decrease during heat treatment of milk above 90°C is, thus, explained by lactose degradation, casein dephosphorylation, and calcium phosphate precipitation. The opposite pH increase during cheese ripening is explained by lactic acid degradation and ammonia production by microorganisms. When milk has been high pressure (HP) treated between 250 and 600 MPa the buffering capacity is maximal at pH 5.2–5.4 compared to pH of 4.8–5.0 for untreated milk. The quantitative importance of this is that micellar calcium solubilisation in pressurized milk was promoted at this higher pH, and more calcium ions were found in milk treated at 600 MPa for 30 min upon acidification to 5.2 compared to untreated

milk [2]. Most likely, the different buffering capacities are a result of different states of colloidal calcium phosphate during the pressurizing process. Indeed, Orlien et al. [3] showed the major influence of the pH of the milk on the barostability of the casein micelles under pressure and explained this by the greater importance of the state of calcium and the associated HP effect on the CCP balance in the micelle-serum system. Furthermore, the pH of the milk has major impact on the extent of  $\beta$ -lactoglobulin ( $\beta$ -Lg) denaturation and it has been showed that HP-induced denaturation of  $\beta$ -Lg decreased at acidic pH but increased at alkaline pH [4, 5].

The pH and HP influences on the physicochemical changes in milk are thus important for the technological aspects of dairy products. However, variations in pH of milk or milk systems during HP-treatment have not yet been elucidated.

In recent years it has been shown that the pH of weak acids changes under pressure due to effect on the ionization equilibrium [6–11]. Depending on the nature of the solution pH may decrease, increase, or remain constant when subjected to high pressure. The pressure dependence of

aqueous solutions is governed by the partial molar volumes under the actual conditions in the equilibrium solutions. Dissociation of acids increases upon pressurizing if the ion-pair formation is accompanied by a substantial volume reduction. Consequently, the buffering capacity of certain buffers decreases with pressure with significant changes in pH upon pressurizing. To our knowledge, only one study on pH changes in protein solution under pressure exists: the pH increased up to 1.5 units in a  $\beta$ -Lg solution at pressure up to 300 MPa followed by a decrease to the initial pH of 4.0 for further pressurization up to 500 MPa [7]. This study was therefore undertaken to monitor the effect on changes in pH of a  $\beta$ -Lg solution at its natural pH and in combination with  $\beta$ -casein ( $\beta$ -CN) during HP-treatment and offers additional information to the understanding of the molecular properties of  $\beta$ -Lg under pressure.

## 2. Materials and Methods

**2.1. Chemicals.**  $\beta$ -lactoglobulin from bovine milk, genetic variant B, was isolated from acidic whey of fresh skim milk of homozygotic cows and purified according to the method described by [12].  $\beta$ -casein-A'A' ( $\beta$ -CN) was a gift from Dairy Technology, Department of Food Science, University of Copenhagen. 3',3''-Dibromothymolsulfonphthalein (bromothymol blue) was obtained from Merck (Germany). All other chemicals were of analytical grade, and solutions were based on highly purified water (Milli-Q Plus, Millipore Corp, Bedford, MA, USA).

**2.2. High Pressure Spectrophotometer.** The intensity of light transmitted through the solutions under investigation of varying pressure was measured *in situ* in a thermostated high pressure optical cell (Type 740.2006 from SITEC Sieber Engineering AG, Switzerland) equipped with a hand-operated pressure generating system (Type 750.1700 from SITEC Sieber Engineering AG, Switzerland) as described by [7]. The pressure generating system and the optical cell were filled with the relevant solution and the intensity spectrum from 350 to 700 nm was recorded at each step of pressures between 0.1 and 500 MPa. The intensity spectra were converted to absorption spectra using  $A_\lambda = -\log(I_\lambda/I_\lambda^{\text{ref}})$ , where  $I_\lambda$  and  $I_\lambda^{\text{ref}}$  are intensities at wavelength  $\lambda$  for the solution and for water as reference, respectively. The intensity spectrum of water was recorded prior to measurement of the indicator solution.

**2.3. In Situ Spectrophotometry.** A stock solution of bromothymol blue ( $3.6 \cdot 10^{-4}$  M) in water was prepared. Solutions with bromothymol blue in its acidic, alkaline, or partially transformed form were prepared by adjusting aliquots to pH 3.0, 10.0, and 7.0, respectively, with appropriate amounts of HCl or NaOH, and to an ionic strength at 0.08 M with NaCl and a final bromothymol blue concentration of  $3.6 \cdot 10^{-5}$  M. Prior to the optical measurements under pressure, the pH of all solutions was measured as a reference with glass electrode (713 pH Meter, Metrohm, Switzerland) against international activity pH standards.  $\beta$ -Lg was dissolved in

water as a stock solution and stored at 5°C overnight for equilibration. The  $\beta$ -Lg solutions for measurement under pressure were made by adding an appropriate aliquot of bromothymol blue (final concentrations of  $3.6 \cdot 10^{-5}$  M) to aliquots of the stock solution giving final  $\beta$ -Lg concentrations of 1.00 and 3.00 mg/mL. The pH of the  $\beta$ -Lg solutions was adjusted with HCl or NaOH to pH 3.0, pH 10.0, and pH 7.0 and to an ionic strength at 0.08 M with NaCl.  $\beta$ -CN was dissolved in water (with 2 mg/mL NaCl) as a stock solution and stored at 5°C overnight for equilibration. The  $\beta$ -CN solutions for measurement under pressure were made by adding an appropriate aliquot of bromothymol blue (final concentrations of  $3.6 \cdot 10^{-5}$  M) to aliquots of the stock solution giving final  $\beta$ -CN concentrations of 1.00 mg/mL. The pH of the  $\beta$ -CN solution was adjusted to pH 7.0 and to an ionic strength at 0.08 M with NaCl. For the mixed  $\beta$ -Lg and  $\beta$ -CN solution, an appropriate volume of  $\beta$ -Lg stock solution was added to the  $\beta$ -CN solution to obtain a concentration of 1.00 mg/mL for both proteins. The solutions were filled in the optical cell of the high pressure spectrophotometer and a series of intensity spectra were recorded and converted to absorption spectra as described above.

The method developed by Orlien et al. [7] was used to monitor *in situ* pH changes in solutions of  $\beta$ -Lg and  $\beta$ -CN and mixture thereof under pressure by measuring the absorption spectra of the respective proteins in solution under acid and basic conditions, and the partially transformed, and calculating the change in pH in accordance with

$$\Delta\text{pH}(P) = \Delta \log \left( \frac{\alpha(P)}{1 - \alpha(P)} \right), \quad (1)$$

where  $\Delta\text{pH}(P) = \text{pH}(0.1 \text{ MPa}) - \text{pH}(P)$ . The degree of dissociation,  $\alpha(P)$ , of the indicator is calculated by

$$\alpha(P) = \frac{A_x(P) - A_a(P)}{A_b(P) - A_a(P)}, \quad (2)$$

where  $A(P)$  is the absorbance of the partially transformed indicator (index  $x$ ) and absorbance of the indicator in its acidic form (index  $a$ ) and in its basic form (index  $b$ ), respectively, at the respective pressure.

**2.4. Statistical Analysis.** The statistical analysis is carried out in R (R version 1.12.1) with RKWard (Version 0.5.4, KDE version 4.5.1) as graphical user interface used for script markup and piping to the Rterminal. The following add-on packages were also used: MASS (Version 7.3-7), nlme (Version 3.1-97), and gmodels (Version 2.15.0).

## 3. Results and Discussion

$\beta$ -Lg is a compactly folded globular protein and consists of 162 amino acid, where 53 residues have titratable side groups [13].  $\beta$ -Lg is a dimer at pH 7 and each monomer has 2 ionisable histidine side groups. However, the two histidine residues differ considerably in solvent accessibility due to the conformation of the protein molecule. As the solvent-accessible area of His146 is around  $126 \text{ \AA}^2$  at pH 7 it is

available to titration, while, in contrast with the solvent-accessible area of His161 around  $12 \text{ \AA}^2$ , it becomes buried in the interior of the native protein [14]. The effective  $pK_a$  of ionisable side groups in a protein depends on different molecular microenvironments and may have either higher or lower value than the respective free amino acid; thus, the  $pK_a$  of His146 is reported to vary from 6.2 to 7.7 and the  $pK_a$  of His161 is reported to vary from 5.8 to 8.5 [13].

Spectrophotometric measurement of pH with acid-base indicators is based on differences in absorption spectra between the acidic form and the basic form of the indicator molecule and the useful range depends on  $pK_i$  [7]. Previously, we have developed a self-consistent method for measurement of changes in pH with pressure based on the fact that the indicator  $pK_i$  is insensitive to pressure and that the indicator molecule does not bind to the protein [7]. A number of possible indicators (neutral red, phenol red, and bromothymol blue) were investigated in detail over the spectral range 350–700 nm in solutions of their acidic and basic forms and of a mixture around  $pK_i$  value of each indicator, since these indicators were considered good candidates for pressure insensitive indicators. Bromothymol blue was chosen for the investigation of pH changes of the  $\beta$ -Lg solutions under pressure due to the sufficient difference in the absorption spectra for the acid, base, and partially transformed forms. Figure 1 shows the absorption spectra for bromothymol blue in aqueous solutions of  $\beta$ -Lg at different pH as a function of pressure. The increase in molar absorptivity upon pressurizing reflects the concentration increase with pressure as the system is compressed. The small variation in absorption spectra of the acid form with pressure is most likely a combined effect of compressibility of the solvent, of deformation of windows of the high pressure cell, and to a much lesser degree of conformational changes of the indicator. As seen in Figure 1, the clear distinction between the acid and basic form ensures an accurate calculation of the degree of dissociation at each pressure [7]. This distinction is emphasized since the changes of the absorption spectra during pressurization reflect the shifted equilibrium,  $\text{HIn} + \text{H}_2\text{O} \rightleftharpoons \text{In}^- + \text{H}_3\text{O}^+$ , according to the spectral changes of bromothymol blue in water at the relevant pHs (data not shown) and, thus, function as a sensor to probe the result of a diffusion controlled transfer of protons from the solvent to the acidic and basic side groups of the protein in contact with the indicator. At initial pH of 7 and pressure (0.1 MPa) the two absorption bands at 431 and 619 nm confirm that bromothymol blue is in a partially transformed form, yet mostly in its acidic form (Figure 1), reflecting that the two histidine residues are between being protonated and deprotonated according to the rather large span of their respective  $pK_a$ . Upon pressurizing, the absorption spectra of bromothymol blue change corresponding to changes in the acid/base equilibrium of the indicator, and as seen the intensity of both absorption bands increased when the pressure increased to 500 MPa.

$\beta$ -Lg is the most abundant whey protein and is used in many applications for its various functional properties,

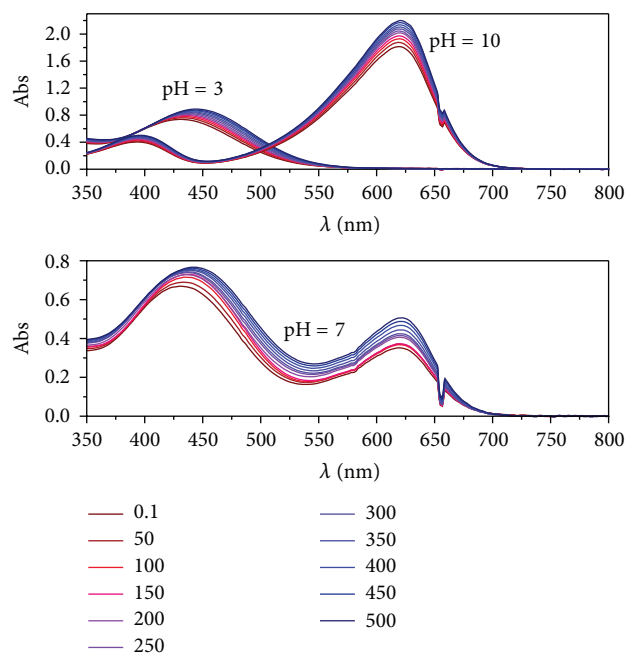


FIGURE 1: Pressure-induced changes in the visible absorption spectra of bromothymol blue in an aqueous solution of  $\beta$ -Lg B (1.00 mg/mL) at acidic (pH 3.0) and basic (pH 10.0) conditions compared to the partially transformed form at pH 7.0. Pressure level in MPa is given by the legend. The abrupt decrease in absorbance at 650 nm is due to the deuterium lamp in the spectrophotometer.

which depend on the physicochemical state and pH. Another group of milk proteins that contributes to the milk buffering capacity is the caseins, and it is reported that the pure caseins have maximal buffering capacity around pH 5–5.5 due to phosphoserine and histidine residues [1]. Moreover, the different genetic variants have different physicochemical properties and different interacting behaviour with other milk constituents depending on the solution conditions.  $\beta$ -CN is the most hydrophobic but also highest charged casein (–13 at pH 6.6) due to an unevenly distribution of hydrophobic (a long C-terminal without any charged side groups) and hydrophilic (a short N-terminal with all charged side groups) residues resulting in a distinct amphipathic character. This unique characteristic makes  $\beta$ -CN very useful for food manufacturers and is one of the most abundant proteins in various food products. The caseins have little tertiary structure and can barely be denatured, but it has been found that, at the  $\beta$ -Lg :  $\beta$ -CN molar ratio of 1 : 0.13 or greater,  $\beta$ -CN is able to suppress the heat-induced aggregation of  $\beta$ -Lg [15]. Figure 2 shows the absorption spectra for bromothymol blue in aqueous solutions of  $\beta$ -CN as a function of pressure and the absorption spectra change considerably with a marked increase in the basic absorption band upon pressurization. With  $\beta$ -Lg in the solution the increase in the basic absorption band was less marked (Figure 2).

From the spectral data and using spectra of the acidic and basic  $\beta$ -Lg solutions (Figure 1) as the acidic and basic form of bromothymol blue, respectively, in the calculation of  $\alpha(P)$

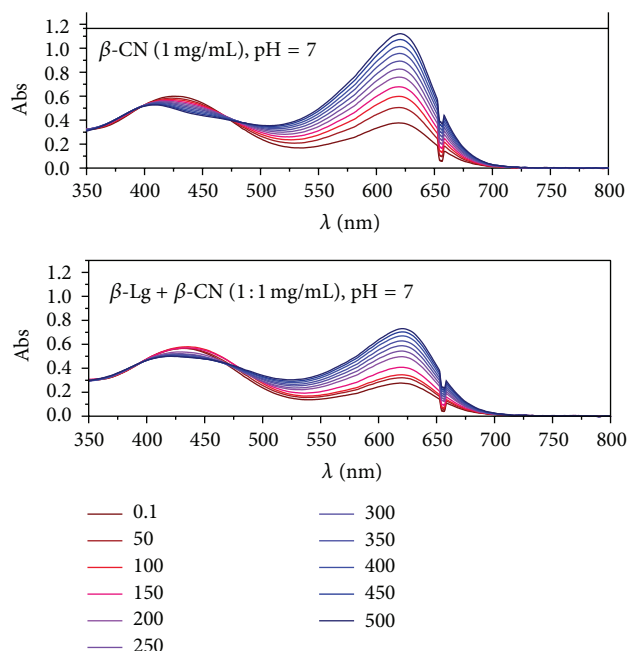


FIGURE 2: Pressure induced changes in the visible absorption spectra of bromothymol blue in an aqueous solution of  $\beta$ -Lg B (1.00 mg/mL) and of  $\beta$ -Lg B +  $\beta$ -CN (1:1 mg/mL) at the partially transformed form at pH 7.0. Pressure level in MPa is given by the legend. The abrupt decrease in absorbance at 650 nm is due to the deuterium lamp in the spectrophotometer.

(2) the change in pH was calculated according to (1) and the results are presented in Figure 3.

The major factors that control the impact of HP on the molecular structure is the electrostriction of charged and polar groups, elimination of packing defects, and the solvation of hydrophobic groups. Pressure treatment was found to induce changes in the pH of the  $\beta$ -Lg aqueous solutions dependent on the working pressure and the minimum in pH was found to be around 150 MPa (Figure 3). The pH profile observed for  $\beta$ -Lg with an initial pH 7.0 (Figure 3) is opposite to the observed pressure dependency for  $\beta$ -Lg with an initial pH of 4.0 [7] and reflects the difference in the titratable sites at the respective pH (histidine versus aspartic and glutamic acid) and in the titration behaviour during pressurization due to conformational changes (extent of residues being exposed to solvent versus buried). From Figure 3 it is seen that high pressure of an unbuffered aqueous solution of  $\beta$ -Lg with an initial pH of 7 induces an immediate decrease in pH up to 150 MPa followed by an abrupt increase in pH beyond the initial pH following a gradual increase to a pH around 7.3 at 500 MPa. Several investigations of high pressure effects on  $\beta$ -Lg have been carried out and several models for the resulting conformational changes have accordingly been suggested. The study of denaturation of  $\beta$ -Lg in skim milk leads to the overall reaction scheme, dissociation of the dimer to monomers, unfolding of the monomeric (still) native structure, and irreversible aggregation with  $\beta$ -Lg or caseins [16]. The pressure denaturation of purified  $\beta$ -Lg was earlier described as a three-step process including an

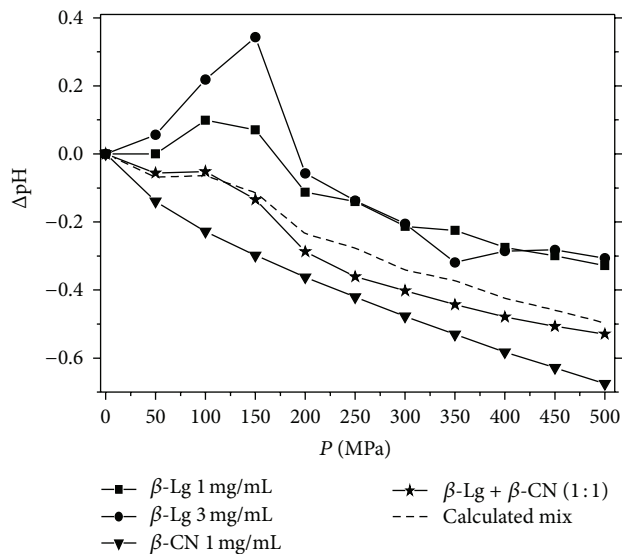


FIGURE 3: Pressure dependence of pH of aqueous solutions of  $\beta$ -Lg B at 1.00 mg/mL ( $\blacksquare$ ) and 3.00 mg/mL ( $\bullet$ ),  $\beta$ -CN 1.00 mg/mL ( $\blacktriangledown$ ), and  $\beta$ -Lg B +  $\beta$ -CN (1:1 mg/mL) ( $\star$ ), all with an initial pH of 7.0. The change in pH,  $\Delta\text{pH}(P) = \text{pH}(0.1 \text{ MPa}) - \text{pH}(P)$ , is calculated from spectral data according to (1). The dashed line represents the  $\Delta\text{pH}$  calculated from the changes of  $\beta$ -Lg (1.00 mg/mL) and  $\beta$ -CN (1.00 mg/mL) according to (3).

initial pressure-melted state for pressure up to 50 MPa, a reversible denaturation up to 200 MPa, and an irreversible denaturation above 200 MPa [17]. Likewise, a three-stage denaturation model for purified  $\beta$ -Lg was suggested with three discernible structural stages: stage I (up to 150 MPa) is the native, stable structure, in stage II (200–450 MPa) the native monomers are reversibly interchanged with nonnative monomers and disulfide-bonded dimer, and in stage III (over 500 MPa) unfolded monomers and dimers interact to form aggregates [18]. The pressure-induced structural changes are, as emphasized by Anema [16], a complex series of more consecutive and/or concurrent pathways than the three mentioned general steps, dependent on conditions like solvent, pressure duration, and temperature. Thus, the HP effect on the structure of  $\beta$ -Lg could be brought together: dissociation into monomers, various molten globule structures, denaturation, and aggregation. It was shown that, upon these HP-induced conformational changes, charged residues undergo a change from buried to exposed leading to an unexpected pH variation of a  $\beta$ -Lg solution under pressure [7]. The pH profiles in Figure 3 show that the initial step where dimers are dissociated into monomers at low pressure of 50 MPa results in no (1 mg/mL) or minor (3 mg/mL) changes in pH of the solution. The following transformation into molten globules is identical to the changes in the  $\beta$ -Lg structure in aqueous solution with initial pH of 4.0 under increasing pressure [7], despite being with a different effect on solution pH. The initial dissociation increases the accessible surface area resulting in an increase in hydration of the protein molecules, resulting in the contraction of solvent water (due to electrostriction), and leading to volume decrease and disruption of ion pairs

in the protein molecule. The  $pK_a$  for histidine residue 146 is 7.5 and 6.8 for the dimer and monomer, respectively, while  $pK_a$  for histidine residue 161 is 6.5 and 5.8 for the dimer and monomer, respectively [13]. Hence, the pH of the  $\beta$ -Lg solution under pressure depends on whether the imidazole rings are protonated or deprotonated. According to the equilibrium  $\text{HisH}^+ + \text{H}_2\text{O} \rightleftharpoons \text{His} + \text{H}_3\text{O}^+$  at the initial pH before pressurisation, most of the His146 was protonated and most of the His161 was deprotonated.  $\beta$ -Lg dissociated from dimer to monomer at the initial increase of pressure and the His146 became available for solvent and shifted to the deprotonated state (due to lower  $pK_a$  for the monomer), resulting in a decrease in pH. During pressurisation up to 150 MPa the dimers are dissociated and provide pathways for water to penetrate into the interior of the monomers leading to the molten globule states. In particular, the dissociation at the dimerization area resulted in an increased accessibility of His161. When the remaining protonated His161 experiences the solvent pH (still higher than the  $pK_a$  for the monomer), it became deprotonated resulting in further decrease of pH. The pressure and solvent pH effects are optimally balanced at 150 MPa, where the maximum decrease in pH occurs (Figure 3). At further increase in pressure the tertiary structure of  $\beta$ -Lg is disrupted leading to a denatured protein making both histidine residues available for the solvent water. As a result of gradual water penetration and the accompanying electrostriction of water, the deprotonated His residues will reassociate the proton from water, which will lead to an excess amount of  $\text{OH}^-$  in the solution corresponding to an increase in pH as seen in Figure 3. As seen in Figure 3 the extent of the pH change is dependent on the concentration of  $\beta$ -Lg, and increasing concentration from 1 to 3 mg/mL brings about a higher amount of histidine residues in effect resulting in a greater pH change. A pH reduction of the observed magnitude (0.34 units for  $\beta$ -Lg at 3 mg/mL at 500 MPa) can be of major importance from a technological point of view. Adjusting the pH of milk by 0.5–0.7 units prior to HP-treatment at 250–600 MPa at 20°C was found to reduce the extent of denaturation of  $\beta$ -Lg considerably compared to milk at normal pH [4, 5]. Interestingly, after reaching a minimum in pH at 150 MPa the pH increased abruptly for both concentrations of  $\beta$ -Lg and increased gradually at further pressurizing.

The nonglobular  $\beta$ -CN is, therefore, insensitive for pressure-induced conformational changes. At the same time it is highly charged and may, therefore, give electrostriction a significant role in the pH behaviour under pressure. The amphiphilic nature of  $\beta$ -CN usually results in self-association into large oligomers/micelle upon dissolving in aqueous media, but the concentration used and the preparation of the solution in this study ensure a solution of  $\beta$ -CN monomers. At 0.1 MPa and pH 7.0 both the 5 histidine residues ( $pK_a \approx 6.5$  [1]) and the 5 phosphoserine residues ( $pK_{a2} \approx 6.3$ –6.8 for phosphoserine residues in  $\beta$ -CN [19]) are shifted to the deprotonated states. Hence, no specific structural rearrangement of the protein will affect solvent pH; only the three “simple” ionisation equilibria related to histidine, phosphoserine, and water self-ionisation will govern the pH change of the  $\beta$ -CN solution under pressure. The underlying

electrostrictive effect is from the water self-ionisation, which is increasingly promoted at increasing pressure, thereby generating charged hydronium and hydroxide ions. It is accepted that exposure and/or generation of polar and charged groups will lead to a decrease in volume due to electrostrictive packing effects. Thus, the  $\text{H}_3\text{O}^+$  ions are reassociated with the deprotonated His and  $\text{Ser-PO}_3^{2-}$  in effect shifting the corresponding equilibria to the protonated states. This will promote a further dissociation of water, which will lead to an excess amount of  $\text{OH}^-$  in the solution corresponding to an increase in pH as seen in Figure 3.

Mixing equal amounts of  $\beta$ -Lg and  $\beta$ -CN resulted in a depressing effect of the individual pressure effects on both proteins, as seen in Figure 3. The initial pH decrease caused by the dimer dissociation and deprotonation of His146 in  $\beta$ -Lg was counterbalanced by the increasing concentration of  $\text{OH}^-$  due to the electrostrictive effect of the  $\beta$ -CN-water system. This finding indicates that the stability of  $\beta$ -Lg under pressure depends on the treatment media and is in agreement with other reports [20, 21]. It was found that  $\alpha_s$ -casein suppressed the pressure-induced aggregation of  $\beta$ -Lg because of the chaperone property of casein [20]. Whether  $\beta$ -CN acted in a similar chaperone manner, thereby reducing the dissociation and denaturation effect, or it was a simple balancing of the pH due to release of  $\text{H}_3\text{O}^+$  and  $\text{OH}^-$  from the  $\beta$ -Lg and  $\beta$ -CN, respectively, cannot be deduced from this study. However, the subsequent pH increase in the solution of the mixed proteins seemed to be an equal contribution of the effect on pH by the individual protein-water systems. Moreover, the pressure course of the pH changes of the mixed solution can be modulated by a simple equation based on the individual HP-pH progress (Figure 3):

$$\Delta\text{pH}(\beta\text{-Lg} + \beta\text{-CN}) = 0.5\Delta\text{pH}(\beta\text{-Lg}) + 0.5\Delta\text{pH}(\beta\text{-CN}). \quad (3)$$

#### 4. Conclusion

The pressure-induced changes in pH of a  $\beta$ -Lg solution at its natural pH was determined by the pressure-induced dissociation and unfolding of  $\beta$ -Lg and the concurrent degree of accessibility of titratable side groups, in this case the two histidine side chains. The importance of the HP effect on the structural changes of the protein and the rearrangement of the protein-water system on solvent pH was supported by the pressure effect on pH in a  $\beta$ -CN solution.  $\beta$ -CN lacks a three-dimensional structure; hence the pressure-induced pH changes were explained purely by the shifts in the equilibria of the histidine and phosphoserine residues as affected by pressure and water's self-ionisation. The HP-induced pH changes in a mixed  $\beta$ -Lg and  $\beta$ -CN solution were found to be a simple mix of the effects from the individual pH profiles under pressure.

#### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

## References

- [1] F. Salaün, B. Mietton, and F. Gaucheron, "Buffering capacity of dairy products," *International Dairy Journal*, vol. 15, no. 2, pp. 95–109, 2005.
- [2] M. H. Famelart, F. Gaucheron, F. Mariette, Y. Le Graet, K. Raulot, and E. Boyaval, "Acidification of pressure-treated milk," *International Dairy Journal*, vol. 7, no. 5, pp. 325–330, 1997.
- [3] V. Orlien, L. Boserup, and K. Olsen, "Casein micelle dissociation in skim milk during high-pressure treatment: effects of pressure, pH, and temperature," *Journal of Dairy Science*, vol. 93, no. 1, pp. 12–18, 2010.
- [4] M. Arias, R. López-Fandiño, and A. Olano, "Influence of pH on the effects of high pressure on milk proteins," *Milchwissenschaft*, vol. 55, no. 4, pp. 191–194, 2000.
- [5] T. Huppertz, P. F. Fox, and A. L. Kelly, "High pressure treatment of bovine milk: effects on casein micelles and whey proteins," *Journal of Dairy Research*, vol. 71, no. 1, pp. 97–106, 2004.
- [6] S. K. Min, P. S. Chaminda, and S. K. Sastry, "In situ measurement of reaction volume and calculation of pH of weak acid buffer solutions under high pressure," *Journal of Physical Chemistry B*, vol. 115, no. 20, pp. 6564–6571, 2011.
- [7] V. Orlien, K. Olsen, and L. H. Skibsted, "In situ measurements of pH changes in  $\beta$ -lactoglobulin solutions under high hydrostatic pressure," *Journal of Agricultural and Food Chemistry*, vol. 55, no. 11, pp. 4422–4428, 2007.
- [8] V. M. Stippl, A. Delgado, and T. M. Becker, "Development of a method for the optical in-situ determination of pH value during high-pressure treatment of fluid food," *Innovative Food Science & Emerging Technologies*, vol. 5, no. 3, pp. 285–292, 2004.
- [9] M. Hayert, J.-M. Perrier-Cornet, and P. Gervais, "A simple method for measuring the pH of acid solutions under high pressure," *The Journal of Physical Chemistry A*, vol. 103, no. 12, pp. 1785–1789, 1999.
- [10] T. K. Hitchens and R. G. Bryant, "Pressure dependence of weak acid ionization in deuterium oxide solutions," *The Journal of Physical Chemistry B*, vol. 102, no. 6, pp. 1002–1004, 1998.
- [11] R. C. Neuman Jr., W. Kauzmann, and A. Zipp, "Pressure dependence of weak acid ionization in aqueous buffers," *The Journal of Physical Chemistry*, vol. 77, no. 22, pp. 2687–2691, 1973.
- [12] K. R. Kristiansen, J. Otte, R. Ipsen, and K. B. Qvist, "Large-scale preparation of  $\beta$ -lactoglobulin A and B by ultrafiltration and ion-exchange chromatography," *International Dairy Journal*, vol. 8, no. 2, pp. 113–118, 1998.
- [13] F. Fogolari, L. Ragona, S. Licciardi et al., "Electrostatic properties of bovine  $\beta$ -lactoglobulin," *Proteins: Structure, Function, and Genetics*, vol. 39, no. 4, pp. 317–330, 2000.
- [14] B. Y. Qin, M. C. Bewley, L. K. Creamer, H. M. Baker, E. N. Baker, and G. B. Jameson, "Structural basis of the tanford transition of bovine  $\beta$ -lactoglobulin," *Biochemistry*, vol. 37, no. 40, pp. 14014–14023, 1998.
- [15] Y. H. Yong and E. A. Foegeding, "Effects of caseins on thermal stability of bovine  $\beta$ -lactoglobulin," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 21, pp. 10352–10358, 2008.
- [16] S. G. Anema, R. Stockmann, and E. K. Lowe, "Denaturation of  $\beta$ -lactoglobulin in pressure-treated skim milk," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 20, pp. 7783–7791, 2005.
- [17] H. Stapelfeldt and L. H. Skibsted, "Pressure denaturation and aggregation of  $\beta$ -lactoglobulin studied by intrinsic fluorescence depolarization, Rayleigh scattering, radiationless energy transfer and hydrophobic fluoroprobng," *Journal of Dairy Research*, vol. 66, no. 4, pp. 545–558, 1999.
- [18] T. Considine, H. Singh, H. A. Patel, and L. K. Creamer, "Influence of binding of sodium dodecyl sulfate, all-trans-retinol, and 8-anilino-1-naphthalenesulfonate on the high-pressure-induced unfolding and aggregation of  $\beta$ -lactoglobulin B," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 20, pp. 8010–8018, 2005.
- [19] J.-J. Baummy, P. Guenot, S. Sinbandhit, and G. Brulé, "Study of calcium binding to phosphoserine residues of beta-casein and its phosphopeptide (1–25) by  $^{13}\text{P}$  NMR," *Journal of Dairy Research*, vol. 56, no. 3, pp. 403–409, 1989.
- [20] J.-S. He, S. Zhu, T.-H. Mu, Y. Yu, J. Li, and N. Azuma, " $\alpha$ s-casein inhibits the pressure-induced aggregation of  $\beta$ -lactoglobulin through its molecular chaperone-like properties," *Food Hydrocolloids*, vol. 25, no. 6, pp. 1581–1586, 2011.
- [21] C. Mazri, L. Sánchez, S. J. Ramos, M. Calvo, and M. D. Pérez, "Effect of high-pressure treatment on denaturation of bovine  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin," *European Food Research and Technology*, vol. 234, no. 5, pp. 813–819, 2012.



**Hindawi**

Submit your manuscripts at  
<http://www.hindawi.com>

